AD_____

Award Number: DAMD17-97-1-7191

TITLE: Monoclonality and Genetic Instability in Premalignant Breast Tissue

PRINCIPAL INVESTIGATOR: Carol L. Rosenberg, M.D.

CONTRACTING ORGANIZATION: Boston University
Boston, Massachusetts 02215

REPORT DATE: January 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

NOTICE

USING GOVERNMENT DRAWINGS, SPECIFICATIONS, OR OTHER DATA INCLUDED IN THIS DOCUMENT FOR ANY PURPOSE OTHER THAN GOVERNMENT PROCUREMENT DOES NOT IN ANY WAY OBLIGATE THE U.S. GOVERNMENT. THE FACT THAT THE GOVERNMENT FORMULATED OR SUPPLIED THE DRAWINGS, SPECIFICATIONS, OR OTHER DATA DOES NOT LICENSE THE HOLDER OR ANY OTHER PERSON OR CORPORATION; OR CONVEY ANY RIGHTS OR PERMISSION TO MANUFACTURE, USE, OR SELL ANY PATENTED INVENTION THAT MAY RELATE TO THEM.

LIMITED RIGHTS LEGEND

Award Number: DAMD17-97-1-7191
Organization: Boston University
Location of Limited Rights Data (Pages): 7-19

Those portions of the technical data contained in this report marked as limited rights data shall not, without the written permission of the above contractor, be (a) released or disclosed outside the government, (b) used by the Government for manufacture or, in the case of computer software documentation, for preparing the same or similar computer software, or (c) used by a party other than the Government, except that the Government may release or disclose technical data to persons outside the Government, or permit the use of technical data by such persons, if (i) such release, disclosure, or use is necessary for emergency repair or overhaul or (ii) is a release or disclosure of technical data (other than detailed manufacturing or process data) to, or use of such data by, a foreign government that is in the interest of the Government and is required for evaluational or informational purposes, provided in either case that such release, disclosure or use is made subject to a prohibition that the person to whom the data is released or disclosed may not further use, release or disclose such data, and the contractor or subcontractor or subcontractor asserting the restriction is notified of such release, disclosure or use. This legend, together with the indications of the portions of this data which are subject to such limitations, shall be included on any reproduction hereof which includes any part of the portions subject to such limitations.

THIS TECHNICAL REPORT HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION.

Patricia D. Madison 11/20/02

_____ _____

REPORT DOCUMENTATION PAGE

OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	January 2000	Annual (1 Jan 99 - 31 Dec 99)

4. TITLE AND SUBTITLE
Monoclonality and Genetic Instability in Premalignant Breast Tissue

5. FUNDING NUMBERS
DAMD17-97-1-7191

6. AUTHOR(S)
Carol L. Rosenberg, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Boston University
Boston, Massachusetts 02215

E-MAIL:
crosenberg@med-med1.bu.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution authorized to U.S. Government agencies only (proprietary information, JAN 00). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

By the time a cancer is detected, its tumor cells already exhibit myriad genetic abnormalities. To gain a better understanding of genetic events that occur earaly in breast carcinogenesis, this research examines genetic abnormalities in 1) histologically normal tissue from women at low, medium or high breast cancer risk, using a variety of archival breast tissues; and 2) in synchronously occuring putative precursor lesions, including normal-appearaing epithelium, simple and atypical proliferative lesions and carcinomas themselves. Each specimen is microdissected, its DNA examined using a panel of selected microsatellite markers and evidence of clonal abnormalities sought, in particular loss of heterozygosity (LOH) and microsatellite instability (MI). Investigation of the project's first goal is generating data regarding the timing and sites of early genetic abnormalities. These data raaise the possibility that a field defect exists in certaain breast tissue. Investigation of the second goal is uncovering that a variety of clonal relationships exist between multiple synchronous putative precursors. These studies should identify important sites of genetic abnromalities in earaly breast cancer precursors, aand begin to outline the sequence of acquired genetic abnormalties needed for precursor lesions to evolve into full-blown malignancies.

14. SUBJECT TERMS		15. NUMBER OF PAGES
Breast Cancer	proliferative, field defect, microsatellite, clonality, loss of heterozygosity	18
		16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Limited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

___ Where copyrighted material is quoted, permission has been obtained to use such material.

___ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

___ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

N/A In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

X For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

X In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

X In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

X In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Carol L Rosenberg 1/14/00

Table of Contents

Introduction:

By the time a cancer is detected, its tumor cells already exhibit a multitude of genetic abnormalities. The goal of this research is to gain a better understanding of genetic events occuring early in human breast carcinogenesis. The research examines genetic abnormalities detectable 1) in histologically normal tissue from women at three different levels of risk for breast cancer; and 2) in synchronously occuring normal-appearing, proliferative (hyperplastic) and malignant lesions.

To execute the research, we are microdissecting numerous histologically-defined epithelial lesions, and non-epithelial control tissues such as lymph nodes or stroma when available, from a series of archived breast tissue specimens. DNA is extracted and amplified by PCR using a panel of between 10-20 microsatellite primers selected for a) relevance to breast tumorigenesis by virture of their location near tumor-suppressor genes implicated in breast tumor development, b) high percent heterozygosity to provide a high degree of informativeness, c) amplified product < 200 bp to be feasible for use in formalin-fixed tissue, and d) ability to be multiplexed together. The amplified products are then examined by autoradiography for evidence of microsatellite changes, in particular loss of heterozygosity (LOH) and microsatellite instability (MI).

The project's first technical objective is to compare the number and sites of abnormalities detected in histologically normal-tissue from women at increasing risk for breast cancer (low risk: reduction mammoplasy, medium-risk: atypical hyperplasia, high-risk: breast cancer already diagnosed). This should provide information regarding the nature of some early genetic abnormalities and address the question of whether a field defect might exist in certain breast tissue. The second technical objective is to investigate the clonal relationship between multiple synchronous putative precursor lesions: normal-appearing epithelium, simple and atypical proliferative (hyperplastic) lesions, and in situ carcinomas. This should help determine common patterns of evolution of precursor lesions from normal-appearing epithelium into the full malignant phenotype.

Body:

Research accomplishments associated with each Task in approved Statement of Work.

Several Tasks were scheduled to be in progress during months 12 –24 of this project. They include:
Task 3: Section Acquisition
Task 4: Microdissection and DNA extraction
Task 5: PCR
Task 6: Analysis of data
Task 7: Interim statistical analysis.

We have been proceeding with these tasks as planned. Details are outlined below:

Task 3: Section Acquisition.

Work over the past 12 months has focused on acquiring tissue specimens (cases) appropriate for the examination of clonal evolution between synchronously occuring lesions (Technical Objective #2). This requires thorough evaluation of pathological materials available for candidate cases to determine if there are normal, proliferative and malignant lesions of sufficient number and size to proceed with microdissection. To obtain 14 cases containing adequate histology, we have had to screen ~ 50. Reasons for cases being inadequate include: insufficient number of normal or proliferative lesions on existing slides, loss of identified lesions after slide cutting, lesions being too small to yield sufficient DNA for analysis. At later stages (see Task 5: PCR) there are also occasional problems with the DNA being too degraded for use. Despite these obstacles, we have successfully examined 191 lesions from 14 independent cancer-containing breast specimens. Every effort has been made to obtain control stromal or nodal tissue, which was mentioned as a concern (Technical Issue b.) in the first Annual Report Review. Table 1, below, details specimen acquisition to date.

Table 1: Breast Specimens: Case ID, Number and Histology of Microdissected Lesions

Case ID:	# Stroma or Nodes	# Normal Epithelium	# Simple Hyperplasia	# Atypical Hyperplasia	# Cancer In Situ	#Invasive Cancer
2004		5	1	3	3	
2008		5		1	2	2
2012		4		4		2
2014	1	1		1	1	
2028	3	7		1	1	
2031	2	5		1	3	2
2034		9		1	1	
2038		5		3	3	5
2039		4	2	8	4	
2052	1	8	1	2	2	3
2053	1	9	2	2	2	1
2070		4		5	3	
2071		9	3	6	2	2
2072	1	10	1	2	2	1
Total #	**9**	**85**	**10**	**40**	**29**	**18**

Acquiring additional specimens for Technical Objective #1 has halted temporarily while investigations on Technical Objective #2 are being actively pursued. The reason is insufficient manpower to pursue both goals simultaneously. Note, however, that numerous cases listed above (i.e., 2004, 2008, 2028, 2031, 2034, 2038, 2052, 2053, 2071, 2072) have sufficient normals to be included eventually in the high-risk group to be studied in Technical Objective #1.

Task 4: Microdissection and DNA Extraction.

Techniques for microdissection and DNA extraction have generally remained the same as in the original proposal. The only exception is we have switched from manual to automated microdissection, using a Laser Capture Microdissection Apparatus (LCMA) (REF). This instrument, developed jointly by the NCI and Arcturus Engineering (Mountain View, CA), uses a narrowly focused pulsed laser to accurately dissect and cleanly remove minute quantities of tissue under direct visualization. The laser "melts" thin tissue sections which then adhere to an Eppendorf cap; the captured tissues can be removed and their nucleic acids and proteins, which are not damaged in the removal process, can be analyzed as needed. The LCMA was purchased as a piece of core equipment by the Department of Medicine and is stationed in the PI's laboratory. Experiments demonstrated that in our own hands there was no difference in the quality of the DNA derived from LCMA obtained specimens vs manually microdissected specimens, (see **Figure 1**). Therefore all microdissections are now performed via LCMA. This speeds things up considerably.

Figure 1: Manual microdissection vs LCM. The left panel demonstrates three separate PCRs from a subject's normal breast epithelium which was manually microdissected. The right panel demonstrates three separate PCRs from a different TDLU from the same subject. Different amounts of template DNA were used in each reaction. The quality of the results is equivalent.

Task 5: Polymerase Chain Reaction (PCR).

The basic technique of PCR has not been altered. Multiplex PCRs using an internal ^{32}PdCTP radioactive label is being performed without alteration from that described in the Statement of Work. The primers being included in the multiplex reaction have undergone only minimal changes, dictated by research results. For instance, at the time of the original proposal, 9 microsatellite markers were included in the multiplex combinations, currently we are using 20 multiplexed into 5-6 reactions (see Table 2). This permits more information to be obtained from the same amount of DNA.

Table 2: Expanded Marker Panel (n=20): Chromosomal Sites and Type of Repeat

Chromosomal Site	Marker	Repeat Type
1q32-42	D1s549	tetra
	D1s213	di
3p24	TGFβ2R	mono
	D3s1283	di
6q26-27	IGF2R	mono
7q31	D7s486	di
11p15	THO1	tetra
	D11s2071	di
11q13	PYGM	di
11q23	D11s1818	di
	D11s1819	di
16q22-24	D16s402	di
	D16s413	di
	D16s512	di
17p13.1	TP53	di
	D17s796	di
	D17s525	di
17q21	D17s1290	di
	D17s579	di
Xq11-12	AR	tri

Task 6: Analysis of Data.

a) Analysis of Data from Technical Objective #1. In collaboration with our statistical consultant (a co-author on our pilot study [1]), we have performed additional statistical analyses of the individuals studied so far. We believe this addresses some of the concern raised in Technical Issue c in the Annual Report Review. These analyses are presented in **Table 3** (below). We reported the contrast between the RM subjects and the breast cancer subjects due to our *a priori* hypothesis that these two groups would differ. For this comparison, the two-sided Fisher's exact p value is 0.076 and the one-sided is 0.071. Because of the small numbers, we also performed an exact analysis to evaluate whether the proportion of women who had at least one abnormality was similar across all 3 groups. A summary of the results from SAS, which uses the methods of Mehta [2] to obtain an exact p value, similar to Fisher's exact, is presented below. The chi-square p value is 0.084 and is inappropriate due to the small sample size. The exact p value is 0.107 for these data, which is suggestive given the small sample size. We also calculated the proportion of abnormal alleles and the proportion of abnormal ducts for each subject. Since each subject had a different number of alleles and ducts examined, we performed an analysis of variance to evaluate whether the average proportions among women differed across the three groups weighting by the number of alleles and ducts respectively. These calculations yielded the results reported in the last two columns of **Table 3**. Again, we focused on the contrast between breast cancer subjects and the RM subjects due to our a priori hypotheses regarding these two groups.

Table 3:

Frequency / Col Pct	Reduction Mammo	Hyper plasia	Breast CA	Total
None	4	2	0	6
	66.67	66.67	0.00	46.15
1 or more	2	1	4	7
	33.33	33.33	100.00	53.85
Total	6	3	4	13
	46.15	23.08	30.77	100.00

STATISTICS FOR TABLE OF POS BY GROUP

Statistic	DF	Value	Prob
Chi-Square	2	4.952	0.084
Fisher's Exact Test (2-Tail)			0.107

```
Sample Size = 13
WARNING: 100% of the cells have expected counts less
          than 5. Chi-Square may not be a valid test.
```

We believe that these results are suggestive but only preliminary. For more definitive analyses, we look forward to accumulating additional subjects and thereby improving the confidence in our results.

In addition, we reexamined the likelihood that the distribution of abnormalities we found could be due to chance. We noted 28/35 (80%) abnormalities were at 4 markers located at sites believed important in breast cancer development: 7q31, 11p15, 17p13, 17q21 [3-8]. This result is not likely due to chance, as the increased occurrence of these abnormalities at these sites in comparison with the other 5 sites is statistically significant ($p < 0.01$) (Fisher's exact test). Although these 4 markers were selected because of their chromosomal location, their overrepresentation among all abnormalities indicates that mutations near these sites may predispose to the formation of genetically aberrant clonal populations. In contrast, mutation at arbitrary or neutral sites may not confer a growth advantage, and a detectable mutant clone may not arise. This suggests that the genetic alterations detected are less likely to be random changes and more likely to be relevant to the earliest stages of breast cancer development.

b) Analysis of Data from Technical Objective #2.

To record data generated from investigation of this objective we have designed a spreadsheet. A sample (case 2014) is provided in the Appendix.

Anlaysis of data from the 14 subjects studied so far reveals several patterns of abnormalities:

1) proliferative lesions, particularly AH, are clonally related to carcinomas as shown below in **Figure 2**.

Case 2014:

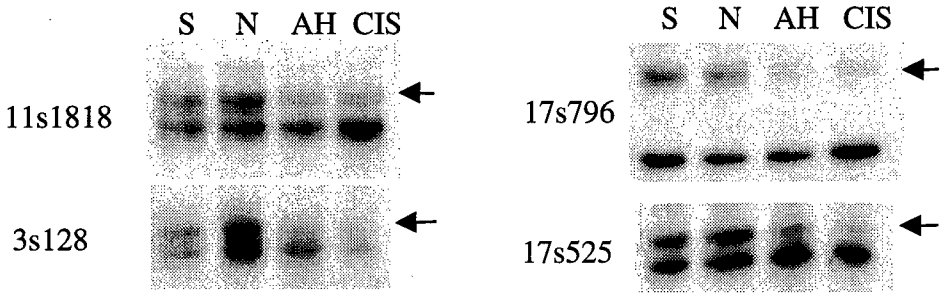

Figure 2: Clonal relationship between proliferative lesion (AH) and carcinoma (CIS) in case 2014. LOH at one allele of each of 4 separate markers is present in the AH and CIS lanes, but not in normal breast epithelial (N) or stromal (S) tissue from the same specimen, as demonstrated by the lighter intensity of the upper band compared to the lower (see arrows). Markers are indicated at the left side of each panel.

2) proliferative lesions, particularly AH, do not share abnormalities seen in synchronous cancers, as shown below in **Figure 3**.

Case 2072:

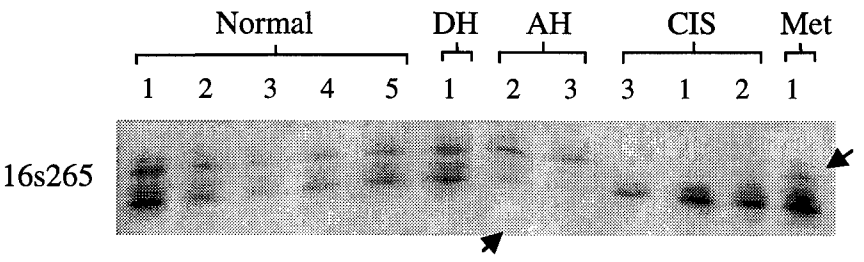

Figure 3: Distinct LOH in proliferative lesions compared with cancers. The normal allelic pattern at this marker is seen in multiple samples of histologically normal breast epithelium and in a simple ductal hyperplasia. Two samples of AH have LOH of the lower allele (indicated by arrow). In contrast, 3 samples of CIS and a metastatic lymph node all have LOH of the upper allele (indicated by arrow).

3) normal appearing tissue can contain abnormalities not seen in synchronous cancers, as shown below in **Figure 4.**

Case 2039:

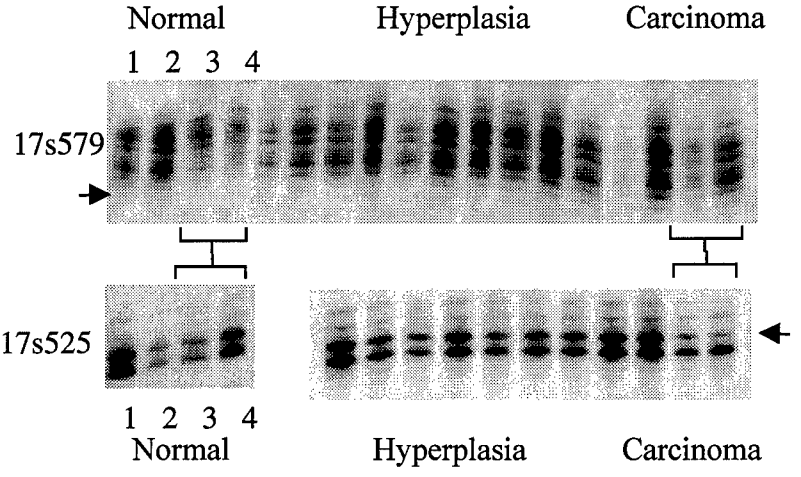

Figure 4. LOH in normal-appearing epithelial ductolobular tissue from cancerous breasts is not seen in synchronous hyperplastic or carcinoma lesions. Normal ducts 3 and 4 both have LOH of the lower allele of marker 17s579 (indicated by arrow in top panel), but none of the carcinoma specimens demonstrate the same LOH. In contrast, 2 carcinoma specimens demonstrate LOH of the upper allele of marker 17s525 (see arrow in bottom panel), but normal-appearing and proliferative lesions do not share that abnormality.

Task 7: Interim Statistical Analyses:

Interim statistical analyses of Technical Objective #1 is reported under Task 6 (Analysis of Data) above.

Key Research Accomplishments:

- Identification 14 independent cases of archival tissue containing synchronous normal, proliferative and malignant breast tissue; identification and microdissection of multiple discrete lesions from each specimen; DNA extraction and fingerprinting of each lesion; creation of spreadsheet to organize data.

- Analysis of data generated from the experiments above indicate several distinct patterns of DNA alterations in premalignant breast tissue: 1) proliferative lesions, particularly AH, share abnormalities with synchronous carcinomas. 2) proliferative lesions and even normal-appearing tissue may contain abnormalities unrelated to cancers occuring synchronously in the breast.

- Continued, refined, statistical analysis of results reported in first annual report continue to validate the findings of increased abnormalities in histologically normal tissue from women at increased risk of breast cancer. This has prompted an application for funding to explore further this observation in different risk groups.

Reportable Outcomes:

Manuscripts:
1. Genomic Instability and Clonal Progression of Premalignant Breast Cancer Precursors. PS Larson, A de las Morenas, **CL Rosenberg**. Manuscript in preparation.

Abstracts:
1. Clonal Progression of Premalignant Breast Cancer Precursors. Larson PS, de las Morenas A, **Rosenberg CL**. Proceedings AACR, 4/2000 #1603. (see appendix)

Funding applied for based on work supported by this award:
NIH R01CA81078 (application pending).

Conclusions:

We have drawn three preliminary conclusions from these data.

1. AH lesions are commonly, but not always, cancer precursor lesions. Another possibility is that proliferative lesions and carcinomas may share a common precursor.

2. Multiple distinct genetically abnormal clones can exist simultaneously. These can appear as proliferative lesions or as histologically normal epithelium and can be clonally distinct from synchronous cancers. Thus, considerable genetic instability and heterogeneity exists in at least some pre-malignant breast epithelium. This may contribute to the genetic heterogeneity documented in breast carcinomas, and to the increased risk of second breast cancers in women already diagnosed with the disease.

3. Continued analysis of these and additional samples should help delineate the extent of this instability, both geographically within a single breast and as a proportion of cancerous breasts. Further studies should also indicate specific genetic abnormalities likely to be critical to early steps in tumorigenesis and associated with progression from premalignant or proliferative lesions to carcinoma, in contrast to abnormalities that may represent more neutral or random mutations. These future studies may also have implications for risk-assessment in women who have not yet developed a cancer, and implications for treatment in women who have already developed a cancer.

References:

1. Larson, P.S., de las Morenas, A., Cupples, L.A., Huang, K. & Rosenberg, C.L. Genetically abnormal clones in histologically normal breast tissue. *American Journal of Pathology* **152**, 1591-1598 (1998).
2. Mehta, C.R. & Patel, N.R. A network algorithm for perforoming Fisher's exact test in r X c contingency tables. *J Am Stat Assoc* **78**, 427-434 (1983).
3. Kerangueven, F. *et al.* Loss of heterozygosity in human breast carcinomas in the ataxia telangiectasia, Cowden disease and BRCA1 gene regions. *Oncogene* **14**, 339-347 (1997).
4. Gudmundsson, J. *et al.* Loss of heterozygosity at chromosome 11 in breast cancer: association of prognostic factors with genetic alterations. *British Journal of Cancer* **72**, 696-701 (1995).
5. Munn, K., Walker, R., L, M. & Varley, J. Allelic imbalance in the region of the BRCA1 gene in ductal carcinoma in situ of the breast. *British Journal of Cancer* **73**, 636-639 (1996).
6. Champeme, M.-H., Bieche, I., Beuzelin, M. & Lidereau, R. Loss of heterozygosity on 7q31 occurs early during breast tumorigenesis. *Genes Chromosomes & Cancer* **12**, 304-306 (1995).
7. Li, L., Li, X., Franke, U. & Cohen, S.N. The TSG101 tumor susceptibility gene is located in chromosome 11 band p15 and is mutated in human breast cancer. *Cell* **88**, 143-154 (1997).
8. Tseng, S.L. *et al.* Allelic loss at BRCA1, BRCA2 and adjacent loci in relation to TP53 abnormality in breast cancer. *Genes, Chromosomes & Cancer* **20**, 377-382 (1997).

Appendix:

I. Sample spread sheet for recording data from Technical Objective #2.

2014

Chr Site	Loci	S1	N1	AH1	CIS1	
1p36	468					x = technical difficulty
						o = heterozygous, no loss
1q32-42	549	ni	ni	ni	ni	• = LOH
	213	o	o	o	o	~ = primers not used
						nl = normal pattern
3p24	TGFbR2	~	~	~	~	ni = not informative
	1283	o	o	•U	•U	
6q26-27	IGF2R	ni	ni	ni	ni	
7q	486	x	?	?	x	AH and CIS are unscorable
11p15	THO1	o	o	o	o	
	2071	o	o	o	o	
11q13	PYGM	o	o	•B	•B	
11q22-23	1818	o	o	•U	•U	
	1819	ni	ni	ni	ni	
16q21	265					
16q22-24	402	x	?	?	x	AH and CIS are unscorable
	413	ni	ni	ni	ni	
	512	ni	ni	ni	ni	
17p13.1	TP53	ni	ni	ni	ni	
	796	o	o	•U	•U	
	525	o	o	•U	•U	
17q12-21	1290	ni	ni	ni	ni	
q21	579	o	o	o	o	
q21	855					
Xq11-12	AR	ni	ni	ni	ni	
Chr Site	Loci	S1	N1	AH1	CIS1	

II. Abstract submitted

Clonal Progression of Premalignant Breast Cancer Precursors. Larson PS, de las Morenas A, Rosenberg CL. Boston University Medical Center, Boston MA 02118.

The earliest recognized breast malignancies, carcinomas in situ (CIS), contain multiple abnormalities, suggesting that precursor lesions exist. Hyperplastic lesions are candidate precursors, since epidemiological evidence links them to increased breast cancer risk, and genetic data indicate they can contain clonal abnormalities. However, their relation to malignancies remains unknown. To determine whether ductal hyperplasias are precursors of ductal malignancies, we multiplexed ~20 microsatellites, from 9 chromosomal arms, and examined loss of heterozygosity (LOH) in DNA from multiple lesions microdissected from single specimens. From 14 specimens, 83 controls (stroma, node or epithelium), 9 simple hyperplasias, 38 atypical hyperplasias (AH), 27 CIS and 17 invasive carcinoma (IC) samples were examined. We find 1) in 6/14 subjects (43%) one or more AH shares site(s) of LOH with CIS and/or ICs in the same specimen. Other, histologically identical, AH may not contain the same LOH. 2) LOH seen in histologically normal tissues is not always detected in synchronous hyperplastic or malignant lesions. These data suggest 1) AHs are genetically heterogeneous but at least a fraction are clonally related to cancers, either as direct precursors or by sharing a common precursor. 2) Aberrant clones in normal-appearing tissue are not obligate cancer precursors. These results should help define sequences of genetic abnormalities that result in breast cancer development.

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 18 November 2002

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for Grant DAMD17-97-1-7191. Request the limited distribution statement for Accession Document Numbers ADB262448, ADB265603 and ADB282072 be changed to "Approved for public release; distribution unlimited." These reporst should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management